diated by calcium influx via the TRPV4 channel, which involves both an influx of extracellular calcium and a release of intracellular calcium stores. In addition, TRPV4 may be involved in modulating the production or influence of pro-inflammatory molecules such as PGE2 or Il-1 in hypo-osmotic conditions. Because of the unique structure of cartilage, mechanical loading is directly coupled to interstitial osmolarity. Thus, in acting as an osmosensor, TRPV4 may also be acting as a mechanosensor. In addition to the short-term changes in osmolarity with loading, osmolarity of cartilage tissue may also change chronically with arthritis.

A7 TRISTETRAPROLIN: A PROSTAGLANDIN E2-RESPONSIVE BIFUNCTIONAL REGULATOR OF CYCLOOXYGENASE-2 EXPRESSION

A.D. Mancini, J.A. Di Battista. *McGill University, Montreal, QC, CANADA*

Purpose: Prostaglandins (PGs) are phospholipid-derived, hormone-like molecules that serve as homeostatic bioregulators and immune and inflammatory modulators. The rate-limiting enzyme in inducible PG biosynthesis is cyclooxygenase-2 (COX-2). Prostaglandin E2 (PGE2), the major COX-2 catalytic product, serves as a feedback regulator of COX-2 (and other pro- and anti-inflammatory) gene expression. Exogenous PGE2 increases primary human synovial fibroblast (HSF) COX-2 mRNA stability and translation; these effects are dependent on p38 MAPK-responsive cis-acting adenylate/uridylate-rich elements (AREs) in the COX-2 3′-untranslated region (3′UTR).
This study's purpose is to characterize the molecular factors (i.e., ARE binding proteins, AUBPs) and mechanisms by which PGE2 increases COX-2 mRNA stability and translation in HSFs.
Methods: Gene reporter studies were conducted to screen three AUBPs expressed endogenously in HSFs and shown to bind the COX-2 3′UTR (i.e., AUF-1, HuR, Tristetraprolin (TTP)) for their effect on COX-2 mRNA stability/translation. HSFs were cotransfected with a luciferase (Luc)-COX-2 3′UTR reporter construct and one of three expression plasmids coding for a different AUBP. Luc activity was then quantified by luminometry as a measure of both mRNA stability and translation; results were expressed relative to Luc activity in cells transfected with an empty AUBP vector (i.e., control). AUBP mRNA and protein expression was analyzed by Northern blotting (NB) and Western blotting (WB), respectively. Subcellular localization studies were performed using real-time confocal microscopy of TTP-GFP-transfected HeLa cells; results were confirmed biochemically by WB.
Results: TTP had the most marked and unambiguous effect on Luc-COX-2 3′UTR reporter activity in HSFs (55±15% decrease in Luc activity). HSF TTP mRNA levels were rapidly (20 min) and potently (3.5-fold vs. control) induced by Il-1β, but displayed a short half-life (1−2 h). Neither Il-1β-induced TTP transcription nor TTP mRNA decay was mediated by PGE2. Interestingly, three TTP transcripts of 6.0, 4.0 and 2.2 kb were detected by NB, suggesting the possible existence of additional TTP isoforms and/or splice variants. In support of this possibility, WB analysis of HSF TTP revealed the presence of a second TTP immunoreactive protein (TTP2, ~60 kDa). Exogenous PGE2 almost completely abolished Il-1β-induced TTP2 protein levels after 17 h of stimulation while minimally reducing the levels of the known TTP protein (TTP1). To verify if TTP2 is a TTP isoform or splice variant, NB analysis of TTP mRNA was conducted using nuclear and cytosolic RNA extracts; only the 2.2 kb TTP transcript was detected in the cytosol, thus indicating that TTP2 is a post-translationally modified form of TTP1. Analysis of TTP's subcellular localization in HSFs and HeLa cells revealed a predominantly (95%) cytosolic localization. PGE2 promoted TTP's nuclear export within 5 min; WB analysis revealed that TTP2 is the shuttling species. The presence of TTP2 in the nucleus and the fact that it is a zinc-finger protein led us to assess its capacity to influence COX-2 gene transcription. Gene reporter studies revealed that overexpression of TTP could transactivate the COX-2 promoter by ~2-fold.
Conclusions: TTP2 is a novel, uncharacterized post-translational variant of TTP1. Although the exact molecular composition and function of TTP2 are unknown, it appears that TTP2 exhibits distinct nuclear (i.e., transcription) and cytosolic (i.e., mRNA decay/translation) functions with regards to COX-2 expression. PGE2's influence on TTP2's expression and subcellular distribution make it a strong candidate effector of PGE2-mediated transcriptional and post-transcriptional/translational gene regulation.

A8 MULTI-JOINT RADIOGRAPHIC OSTEOARTHRITIS (rOA) PHENOTYPES AMONG AFRICAN AMERICANS (AA) AND WHITES: THE JOHNSTON COUNTY OSTEOARTHRITIS PROJECT

A.E. Nelson[1], F. Fang[2], J.B. Renner[1], T.A. Schwartz[3], C.G. Helmick[4], J.M. Jordan[1]. *[1]University of North Carolina School of Medicine, Chapel Hill, NC, USA, [2]StatWorks, Inc, RTP, NC, USA, [3]University of North Carolina Department of Biostatistics, Chapel Hill, NC, USA, [4]Centers for Disease Control and Prevention, Atlanta, GA, USA*

Purpose: Racial differences in OA prevalence have been demonstrated at individual joint sites, but whether there are racial differences in patterns of multi-joint OA involvement is unknown. The current study was designed to assess differences in multi-joint rOA phenotypes among AA and White individuals.
Methods: We conducted a cross-sectional analysis using data from the Johnston County Osteoarthritis Project (n = 1600, 67% women, 32% AA). Hand rOA was defined as (1) Kellgren-Lawrence (KL) grade ⩾2 in at least 3 joints, including DIPs, PIPs, or CMC1; (2) at least 2 involved joints in the same joint group; (3) at least one involved DIP in digits 2−5; and (4) bilateral distribution. Tibiofemoral (TFJ) or hip joint rOA was defined as a K/L grade ⩾2; patellofemoral rOA (PFJ) as an osteophyte grade ⩾2; and lumbosacral spine rOA as an osteophyte grade ⩾1 and disc space narrowing at the same vertebral level. Frequencies were calculated for all joint sites. Generalized estimating equations (GEE) were used to investigate racial differences in OA phenotypes simultaneously, adjusting for correlation among joints of the same individual. Logistic regression was used to assess multi-joint rOA phenotypes as separate outcomes (i.e. hand/knee, hand/hip) in a subset of individuals with complete data for rOA at the hand, TFJ, PFJ, hip, and knee (n = 834), with exact methods used for outcomes with small cell sizes. Analyses were also adjusted for age, gender, and BMI.
Results: The mean (SD) age of the sample was 63 (11) years. Overall, 27% of the participants had hand rOA, 33% had TFJ rOA, 49% had PFJ rOA, 30% had hip rOA, and 49% had rOA of the lumbosacral spine. In unadjusted analyses by race, Whites more frequently had any hand rOA (35% vs 11%, p < 0.0001) and rOA of the spine (52% vs 44%, p = 0.03); AAs more frequently had TFJ rOA (39% vs 30%, p = 0.0003); and no racial differences were seen for hip or PFJ rOA. Results were similar in adjusted analyses by race (using GEE) except that there was no racial association for spine involvement. In unadjusted analyses of multi-joint rOA phenotypes, compared to those with no rOA at any site (15% of the subset), AAs had significantly decreased frequencies of isolated hand or PFJ involvement compared to Whites, as well as less frequent combinations of joints including the hands (hand/TFJ, hand/hip, hand/TFJ/hip, Table). After adjustment, AAs were less likely to have any combination of involved joints that included the hands (hand only, hand/TFJ, hand/hip, or hand/TFJ/hip) compared to Whites. AAs had 50% increased odds of TFJ and hip involvement together, but this did not reach statistical significance (Table).

Table 1: Unadjusted frequencies and adjusted odds ratios for multi-joint rOA phenotypes, by race

Joint site(s)	AA *n* = 231 (%)	White *n* = 603 (%)	Unadjusted p value*	Adjusted OR (95% CI)**
None	50 (22)	78 (13)		
Hand only	0 (0)	33 (6)	**<0.001**	**0.05 (0−0.32)**†
TFJ only	19 (8)	22 (4)	0.409	1.45 (0.64−3.28)
PFJ only	35 (15)	116 (19)	**0.004**	0.61 (0.35−1.07)
TFJ/PFJ only	10 (4)	10 (2)	0.354	1.59 (0.56−4.48)
Hip only	11 (5)	29 (5)	0.184	0.72 (0.30−1.71)
Hand/TFJ	20 (9)	114 (19)	**<0.001**	**0.28 (0.14−0.57)**
Hand/hip	4 (2)	23 (4)	**0.016**	0.34 (0.07−1.25)†
TFJ/hip	58 (25)	77 (14)	0.520	1.52 (0.88−2.61)
Hand/TFJ/hip	24 (10)	101 (17)	**0.001**	**0.40 (0.19−0.85)**

*p value for AA vs White, unadjusted frequencies, **aOR for AA vs White, adjusted for age, gender, and BMI, † by exact logistic model due to small cell count

Conclusions: Multi-joint rOA phenotypes differ by race, with AAs more likely than Whites to have multiple large joint OA involvement. Definitions of generalized OA which emphasize hand involvement, commonly used in Whites, may not identify AA individuals with multi-joint OA.